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# In Situ Latex Agglutination in Seroprevalance of Toxoplasmosis in Pastoral Herds of Goat and Attendants in Premises of Fort Munro Punjab, Pakistan

Shams-ur-Rehman[a], Mansur-ud-Din Ahmad[a], Muhammad Ehsan[b], M.Waqas Hassan[b], Muhammad Ali Memon[b], Naveeda Akhtar Qureshi[c], Abid Ali[c] , Muhammad Haseeb[c*]

[a] Department of Epidemiology and Public health, Faculty of Veterinary Sciences, University of Veterinary and Animal Sciences, Lahore, Pakistan.
[b]College of Veterinary Preventive Medicines, Nanjing Agricultural University, Jiangsu PR, China
[c]Department of Animal Sciences, Quaid-i-Azam University Islamabad 45320, Pakistan

**Abstract**
*T. gondii* is causative agent that responsible one-celled parasite, ''Toxoplasma'', with zoonotic properties, it cause major problem in ovine and caprine family through cat which is act as a carrier. A cross sectional study was conducted on 52 pastoral herds of goats and herd-attendants during March, 2013 to May, 2013 to observe the prevalence and investigate the potential risk factors of Toxoplasmosis in pastoral goats and herd attendants in Fort Munro Punjab, Pakistan. A total of 237 goats from 52 pastoral herds and their 92 male herd-attendants were screened using Latex agglutination test. Overall 27% prevalence was recorded in goats, 69% in pastoral herds and 19.6% in herd-attendants. The prevalence of disease was 14.77% in Pahari and 12.24% in Hairy goat breed whereas 21.52% in female and 5.49% in males. Seropositivity trend was increased with increase in the age of goats and attendants. Natural water reservoirs, presence of cats and dogs in herds, offering raw meat offal to cats and dogs and pastoral grazing itself were significant risk factors of Toxoplasmosis for goats. Risk factors like, medium-cooked meat, eating unwashed fruits and raw vegetables and access of cats to water and food were significantly associated with Toxoplasmosis in herd attendants. It is concluded that disease burden of Toxoplasmosis is increasing gradually and globally. It can be reduce in human beings and animals by reducing risk factors and maintaining proper hygienic events
**Keywords:** *Toxoplasma gondii*, Pastoral goat herds, Seroprevalance, Preventive measures.

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***Corresponding author** Muhammad Haseeb **E-mail:** muhammadhaseeb73@gmail.com

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## Introduction

Toxoplasmosis is a protozoan disease of cats, however, all warm-blooded animals comprising mammals, birds and human beings can be infected with Toxoplasmosis because of its zoonotic and food-borne nature [1, 2]. It is caused by a crescent shape (Greek. Toxo = arc or bow, plasma = creature), ubiquitous, obligate intracellular protozoan called Toxoplasma gondii [3]. Two scientists, Nicolle and Manceaux, in 1908 and 1909, initially discovered it, when they were working on Leishmania in a hamster like rodent, Ctenodactylus gondii [4]. Although it has a widest range of intermediate hosts yet there is only one species; gondii under the genus Toxoplasma [5]. Infection in one-third human population indicates that since the time of its discovery, Toxoplasma gondii is remarkably a successful protozoan that is endemic and distributed worldwide [6].

Goats are important domestic animals in Pakistan. It is considered as a poor man's cow because this species provides sufficient milk for a small family and has a minimum cost of rearing and maintaining. But still Toxoplasmosis in goats is under reported in Pakistan despite the fact it is a major cause of early embryonic death, neonatal lose and abortion in goats [7]. Toxoplasmosis has also serious implications on public health because being a zoonotic and foodborne infection, it can be transmitted to human beings via contaminated meat and milk. Data on human Toxoplasmosis is also insufficient in Pakistan although Toxoplasmosis is life threatening in immunocompromised individuals and is associated to neuro-ophthalmic disorders in apparently healthy persons [8]. During pregnancy Toxoplasmosis leads to serious and fatal disease to the fetus and death of fetus may occur [9]. However, if the fetus survives, Toxoplasmosis is attributed to central nervous disorders, hearing defects, eye disease (ocular toxoplasmosis) and thus adversely affects quality of life of neonates [10].

Being a foodborne, infection Toxoplasmosis is significantly concerned with food safety strategies [11]. It has been recently calculated that Toxoplasmosis has a same disease burden as Salmonellosis and Compylobacterosis [12]. It is considered as a third major cause of food related mortalities in the USA and the cost of treating Toxoplasmosis and Toxoplasmosis related abortions, congenital defects, hearing loss, blindness and mental disorders is seven billion dollars every year [10]. It is ranked as second priority emerging zoonosis in the Holland [13].

Major risk factors of Toxoplasmosis in human beings are undercooked meat and unpasteurized milk of infected animals. Regardless of the fact that no representative data is available about Toxoplasmosis in goats and human beings, a cross sectional study was conducted in Fort Munro Pakistan. The objectives and prime goal of this study was to provide baseline data about the prevalence and risk factors of Toxoplasmosis in goats and herd attendants in the study area.

## Materials and methods

### Locality and Animals

Current study was conducted in premises of Fort Munro, which is an area of Suleiman mountains district D.G.Khan and located at the boarder of Punjab and Baluchistan provinces of Pakistan, Thus a wide variety of temperatures, annual rain fall and has the highest population of goats throughout the Punjab.

### Population density and model size

A total of 237 goats from 52 pastoral herds of goats, with herd size of at least 10-40 animals, were screened during this study. Convenience sampling was used for herd's selection and goats from each herd were selected by systematic sampling, i.e. each 5th unit was selected. All male herd attendants of selected herds were also included in this study. Overall, 92 herd attendants of 52 herds were selected during this study. The sample size for goats was calculated by epidemiological formula, as described by Thrusfield ; n = (1.96)2 P exp. (1-Pexp)/d2 [14].

### Blood sampling and technique

Blood was collected from jugular vein of 237 goats. 2-3 ml blood was taken from the superficial vein of upper limbs of 92 herd attendants by experts and well trained paramedical staff with the help of sterile disposable syringes under sterile and aseptic conditions. The benefits of the study were explained before the herd attendants and verbal consent was taken before taking their blood. All the blood samples were transferred into yellow caped BD vacutainer and were allowed to clot under field conditions. Serum was immediately separated and was transferred into sterile serum cups. All these serum samples were labeled according to species, age, and sex and transported under refrigerator temperature to EPH, UVAS lab, Lahore where all samples were centrifuged at 5000 rpm for 15 minutes in order to obtain clear serum samples. The clear serum samples were then transferred to another sterile serum cups with the help of micropipette and were stored at 20oC until processed for analysis. Repeated freezing and thawing was avoided during processing and analysis of samples.

### Sample analysis

The commercial Latex agglutination kit (Wiener Laboratories S.A.I.C. Riobamba 2944, 2000 Rosario Argentina) was used for detection of specific antibodies of T.gondii in serum of goats and herd attendants. The procedure and protocol were followed according to the manufacturer's instructions and recommendations. Negative 1:16 serum indicated absence of anti T.gondii antibodies. Positive 1:16 serum indicated the presence of anti T.gondii antibodies. Positive titers from 1:32 to 1:128 were due to acquired or evolving immunity. Positive titers equal or higher than 1:256 indicated recent infection.

### Data analysis

Statistical data from questionnaires and lab tests were analyzed by using SPSS version 20 and Graph Pad (PRISM 5). The relationship between prevalence and risk factors was observed by Pearson chi square X 2, probit and logit regression test. While "P" value of <0.05 was considered statistically to be significant.

## Results and Discussion

In From 52 pastoral herds of goats, 36 herds presented at least one seropositive animal. Out of 237 goats, 64 tested were positive for T.gondii antibodies representing overall 27% prevalence of Toxoplasmosis in goats. The two breeds, Pahari and Hairy goats sampled in the study had a prevalence of 14.77% and 12.24% respectively. But the difference between the prevalence of these two breeds was not statistically significant. Herd level prevalence was associated with the composition of the herd. The herds having older animals were more positive as compared to those herd having younger animals. An increased trend of sero-positivity was also observed along with increase in age of animals (Table 1). Male goats had 5.5% prevalence of Toxoplasmosis compared to 21.5% prevalence in female goats. The difference of sero-positivity between male and female was statistically significant. This study represents that "presence of cats and dogs in herds" is a significant risk factor (P=0.014) of Toxoplasmosis in goat herds (Table 2). Drinking water from contaminated "natural reservoirs" is also a significant risk factor (P=0.02) for Toxoplasmosis in goats. However, other factors like; common grazing of herds with other animals and frequent sale or purchase of animals was not significantly associated with Toxoplasmosis. Pastoral grazing itself is also a significant risk factor for Toxoplasmosis. Offering

raw meat offal to cats and dogs present within the herd was also a significant risk factor for Toxoplasmosis in goats. Overall 92 male herd attendants were screened during this study. Female herd attendants could not be included in this study as they refused to participate. Out of 92 herd attendants, 18 attendants were positive against T.gondii antibodies, representing overall 19.6% prevalence in herd attendants. The prevalence of T.gondii antibodies was 10.87%, 5.43% and 3.26% in age groups >40 years, 20-40 years and <20 years respectively (Fig. 1). Only two serum samples, one from age group <20 years and one from 20-40 years showed agglutination at 1:256 dilution of serum, indicating recent infection. Tasting of meat while cooking or medium cooked meat was a significant risk factor (P=0.013) for Toxoplasmosis in herd attendants (Table 4). Access of cats to drinking water and food (P=0.021), Taking meal without washing hands (P=0.044) and eating unwashed fruits and vegetables (P=0.04) were also significant risk factors of Toxoplasmosis. However keeping cats and dogs as a pet or for herd security (P=0.171) and consuming goat raw milk (P=0.150) were not statistically significant risk factors of Toxoplasmosis for herd attendants (Table 3). However, Toxoplasmosis is more prevalent in Kajli (Pahari) breed as compared to hairy goat breed. The prevalence of Toxoplasmosis in Kajli breed is 14.8% and in hairy goat breed is 12.2% (Fig. 2).

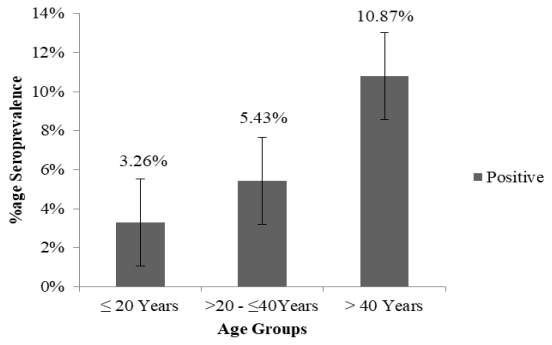


**Fig. 1**: Seroprevalence of Toxoplasmosis in male goat's herd attendant

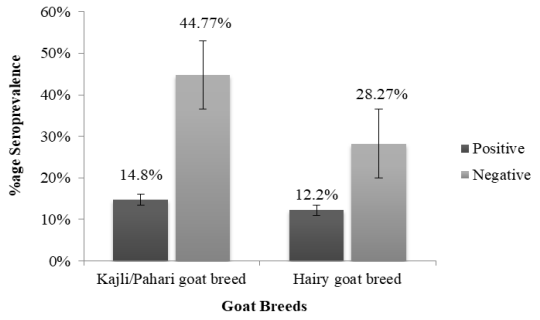


**Fig. 2**: Prevalence of Toxoplasmosis in goats with respect to goat breeds

In present study commercial latex agglutination kit was used for the sero-diagnosis of Toxoplasmosis in goats and their attendants. LAT is comparatively superior test to other serological tests because of its high sensitivity and specificity [11]. Many researchers from the different parts of the world have reported the prevalence of anti T.gondii antibodies in goats by using different serological tests like ELISA [15], LAT [11], IHA [16], IFAT [17] and MAT [18].

The overall prevalence of T gondii antibodies coincides with Tzanidakis *et al*. 2012, who recorded 30% prevalence in goats in Greece. Other scientists from all over the world have recorded the prevalence of Toxoplasmosis in goats as 31% in Durango state, Mexico [19], 31% in Uganda [20], 30.2% in Poland [21], 24.5% in Patois city, Paraiba state, Brazil. This variation in the seroprevalance may be due to different diagnostic techniques, demographics of goats population, breeding conditions, environmental and manage-mental conditions [22], immune status and timing of infection [23], distribution and population of cats in a particular area [24]. Infections are comparatively more common in hot and moist areas of the world [25]. But the result of the present study signifies that there is also a high prevalence of Toxoplasmosis in goats in mountainous areas. The findings of present study also coincides with the observation of Carneiro *et al*. 2009 and Czopowicz *et al*. 2011, that infection is more prevalent in adult animals as compared to young animals. This study is in line with the observation that "presence of cats and dogs in goat herds" is a significant risk factor of Toxoplasmosis [21]. Another risk factor that found significant in this study was "drinking water from contaminated natural reservoirs". Many researchers have also found that stagnant water bodies are potential risk factors of Toxoplasmosis [15]. Water from public supply is also a risk factor of Toxoplasmosis for goats in Greece [26]. Jones and Dubey 2010 have evaluated that municipal water reservoirs in the Canada are significant risk factors of Toxoplasmosis. The present study indicates that "mutual grazing of herds with other animals like cattle and sheep" is associated with Toxoplasmosis but statistically it is not significant. Many studies from other parts of the world have found that common grazing of goat herds with other animals is a significant risk factor for Toxoplasmosis [11]. The present study is higher prevalence of Toxoplasmosis in females as compared to males. The higher prevalence in females might be the fact that females kept for longer period so they were more exposed to risks as compared to males. Males are frequently slaughtered or sold and few bucks are raised in

**Table 1:** Prevalence of Toxoplasmosis in goats with respect to different age groups

| Age | Parameters | Results |
|---|---|---|
| ≥6 m-<1Year | Number of animals | 10 |
| | Prevalence within Age | 20.8% |
| **Share in overall prevalence** | | **4.2%** |
| ≥1-<3 Years | Number of animals | 25 |
| | Prevalence within Age | 29.1% |
| **Share in overall prevalence** | | **10.5%** |
| ≥3 Years | Number of animals | 29 |
| | Prevalence within Age | 28.2% |
| **Share in overall prevalence** | | **12.2%** |
| **Total** | Number of animals | 64 |
| | **Overall prevalence** | **27.0%** |

This table represnts that age group of "≥3 years" has highest share of percentage prevalence to overall prevalence of Toxoplasmosis in goats, followed by age group "≥1-<3 years", while age group "≥6 m - <1Year" has lowest share of percentage prevalence to overall prevalence of Toxoplasmosis in goats.

**Table 2**: Risk factors of Toxoplasmosis in herds of goats

| Sr. # | Risk factors | Pearson Chi-Square Value | Df | P Value |
|---|---|---|---|---|
| 1 | Presence of cats and dogs in herds | 6.083 | 1 | 0.014 |
| 2 | Mutual grazing of herd with other animals (cattle, sheep) | 2.62 | 1 | 0.105 |
| 3 | Pastoral grazing | 4.93 | 1 | 0.026 |
| 4 | Drinking water of natural reservoirs | 4.90 | 1 | 0.02 |
| 5 | Frequent purchase and sale of animals | 0.085 | 1 | 0.771 |
| 6 | Offering raw meat/offal to cats and dogs present within the herds | 5.255 | 1 | 0.022 |

**Table 1:** Risk factors of Toxoplasmosis in goat/herd attendants

| Serial # | Risk factors | Chi-Square Value | df | P Value |
|---|---|---|---|---|
| 1 | Consumption of goat raw milk | 2.075 | 1 | 0.150 |
| 2 | Tasting of meat while cooking | 6.198 | 1 | 0.013 |
| 3 | Keeping cats or dogs as a pet | 1.875 | 1 | 0.171 |
| 4 | Access of cats to drinking water and food | 5.362 | 1 | 0.021 |
| 5 | Taking meal without washing hand with soap | 4.046 | 1 | 0.044 |
| 6 | Eating fruits and raw vegetables without washing with clean water | 4 | 1 | 0.04 |

herds only for breeding purpose. Another reason for high prevalence of Toxoplasmosis in females may be that females bear more physiological stresses like, estrus, lactation, pregnancy and birth etc. These stresses depress the immunity in females and chances of infection increases. The results of present study indicate that there is no association between the prevalence of disease with frequent sale and purchase of animals. In the present study prevalence of Toxoplasma gondii antibodies was higher in Pahari breed as compared to hairy goat breed. The results of this study signifies that there is no association of Toxoplasmosis with breeds and neither it was statistically significant. Zahu *et al*. 2011, and van de puije *et al*. 2000, found a significant breed difference in the susceptibility of Toxoplasmosis. This difference could be due to difference in the resistance of breeds to parasitic infections.

Overall 19.6% seroprevalence was recorded in herd attendants in present analysis. A study conducted in Tanga district of Tanzania represents 52.2% seroprevalence of Toxoplasmosis in livestock handlers [27] which is very high as compared to present study. Many studies found the prevalence of Toxoplasmosis in human beings like 19.2% in Durango, 11.9% in Amazonia [28], 48.7% in Argentina, 36.9% in Chille, 18.6% in Canada and 11.1% in Manila [29]. A study in the China represents T. gondii infection rate between males and females in the 19-20 years age group is more obvious, with 6.4% prevalence in male individuals and 14.6% prevalence in females [30].

**Table 4:** Distribution of anti-Toxoplasma gondii antibodies in Farm workers using Latex Agglutination test (LAT), in relation to age.

| Age groups | 1:16 | 1:32 | 1:64 | 1:128 | 1:256 | Total |
|---|---|---|---|---|---|---|
| **≤ 20 Years** | - | 1 | 1 | - | 1 | 3 |
| **>20-≤40Years** | - | - | 3 | 1 | 1 | 5 |
| **>40 Years** | - | 2 | 4 | 4 | - | 10 |

During recent study it is observed that "tasting meat while cooking" is a significant risk factor of Toxoplasmosis in goat attendants. Many researchers have found that there is a significant relation between consumption of undercooked meat and Toxoplasmosis [8, 31]. Undercooked or medium cooked meat of infected animals contains tissue cysts which are the potential risk factors of Toxoplasmosis for human beings [32]. However consumption of goat raw milk is more discussable because according to many researchers tachyzoite stage of T gondii secreted along with milk is not

resistant to pepsinogen [33]. Although tachyzoite stage is sensitive to the activity of proteolytic enzymes and frequently destroyed by the action of gastric juice yet it is a great risk factor for new born babies who have a lower concentration of proteolytic enzymes and thus they are more susceptible to Toxoplasmosis as compared to adults [33]. In present study it was observed that Toxoplasmosis has an association with consumption of raw milk but it was not statistically significant. The results of present study show that although presence of cats and dogs in herds is significantly associated with Toxoplasmosis yet this risk factor is not statistically significant in case of goat attendants. This observation suggests that keeping cats and dogs as a pet or for herd security is not a risk factor of Toxoplasmosis for goat attendants. Many scientists have also found that consuming undercooked meat and contact with cat feces is a greater risk factor of Toxoplasmosis rather than contact with cats or dogs [34].

## Conclusions

Keeping all previous experimental facts, it is concluded that disease burden of Toxoplasmosis is increasing gradually and globally. It can be reduce in human beings and animals by reducing risk factors and maintaining proper hygienic measures. It is recommended that meat should always be used after full cooking. Ingestion of raw milk should be discouraged, especially in children. Cats should be kept away from goats or animal herds. As human females could not be included in this study and Toxoplasmosis may also cause abortion in human females so it is further recommended that next studies should be focused on females of childbearing age in the study area. Moreover, studies on meat, meat products, milk and milk products will be helpful to alleviate the disease burden of Toxoplasmosis in human beings.

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The authors declare that there is no conflict of interests.

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